THE MUCOSA OF THE STOMACH OF THE WOMBAT (*VOMBATUS HIRSUTUS*) WITH SPECIAL REFERENCE TO THE CARDIOGASTRIC GLAND

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(Plates III–V)

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Synopsis

The specialised cardiogastric gland region of wombat stomach which is characteristic of koala and beaver stomachs as well, is located on the lesser curve near the oesophageal opening. The cardiogastric gland in the wombat is distinctive because of its complex group of mucosal sacculations which open into the stomach lumen via 25 or 30 large crater-like ostia. The mucosa of this gland contains long, straight, closely packed, unbranched gastric glands composed of the cell types found elsewhere in the stomach, with chief cells concentrated at the base of the glands. Parietal cells are present in great abundance. Typical surface and neck mucous and argyrophilic cells are also present. The bizarre cardiogastric specialisation in the wombat is thus not cytologically a separate organ from the stomach. However, it does contribute greatly to the total secretory cell mass of the stomach.

INTRODUCTION

The stomach of the wombat (*Vombatus*), koala bear (*Phascolarctos*) and beaver (*Castor*) are of unusual anatomical interest due to the presence of a convoluted mucosal specialisation, or cardiogastric gland on the lesser curve, near the oesophageal opening. The first reported observations on the stomach of a wombat were made by Everard Home (1808); since then only brief descriptions of gross anatomical features of the stomach have been published (Oppel, 1896; Mackenzie, 1918; Milton, 1962). Some histological aspects of the cardiogastric gland region were studied by Johnstone (1898), but the techniques of that day did not permit micro-photography of the cytological organisation of the mucosa. This study was undertaken in order to clarify the organisation of this unusual stomach.

MATERIAL AND METHODS

Twenty-four adult wombats of both sexes (species: *Vombatus hirsutus*) were trapped in rural areas of Victoria and New South Wales. The animals were killed and the stomach was removed within five minutes of death, distended with 10% formal saline and immersed in this fixative. One stomach was sectioned and blocks placed in osmic acid-zinc iodide fixative, to stain the postganglionic unmyelinated parasympathetic nerve fibres (Maillet, 1963). Material for light microscopy was embedded in paraffin wax and sections 7–8 microns in thickness were stained by various methods. These included haematoxylin and eosin, periodic-acid-Schiff reaction (PAS), block silver impregnation (Masson, 1928), thionin and methylene blue. An incubation time of 36 hours was found preferable for the block silver impregnation.

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Observations

General Features of Wombat Stomach

The wombat stomach resembles that of the human in shape, and thus the usual regional nomenclature will be used: cardia, fundus, corpus and pylorus. The cardiogastric gland is found immediately distal to the cardiac region, hence its name (suggested by Smith *et al.*, 1911) for the analogous structure of the beaver.

The stomach wall varies considerably in thickness, from 0·6 mm. in the fundic region to 2·0 mm. in the corpus and pyloric region. The cardiogastric gland is the notable exception, as the thickness of the stomach wall in this region varies from 4 to 8 mm. The empty stomach measures 30 to 35 cm. along the greatest length from fundus to pylorus. It is thrown into several longitudinal folds which appear to converge on the cardiogastric gland except at the pyloric border. These disappear when the stomach is distended.

The cardiogastric gland can be identified externally on the lesser curve by several criteria:

(1) Its bulging position just distal to the oesophageal-gastric junction.
(2) A ruddy brown colouration due to the skeletal muscle fibres which run over it from the oesophagus, parallel with the longitudinal axis of the stomach. These fibres terminate, though not abruptly, near the pyloric border of the gland.
(3) A fold of lesser omentum and large stomach vessels which are seen crossing the stomach wall, just distal to the gland. However, the gland borders are not sharply outlined on the serosal surface.

The cardiogastric gland, on the luminal side, is distinguished by 25–30 crater-like outlets into the lumen of the stomach (Pl. III, Figs 1 and 2). These outlets, as shown in Figs 1 and 2 are convoluted within; they are not simple ostia. The trapezoid shape of the gland is well defined by these outlets, although convolutions of mucosa underlie the surface epithelium for a few millimetres peripheral to the outlets. The gland has its axis on the lesser curve; it converges to its narrowest dimension at the oesophageal opening. The mean surface area of these "gland trapezoids" as measured in six wombats was 15·0 cm². The outlets are often missing from the axis itself with only simple ridging present instead. Occasionally an outlet can be seen somewhat peripheral to and isolated from the main mass of the gland.

Regional Organisation of mucosa

The Cardia.—Typical cardiac, or mucus-secreting glands, are present in a narrow ring-shaped area (2 mm.) surrounding the oesophageal opening. Parietal cells begin to appear in the more peripheral part of this band. Only mucous cells are present immediately adjacent to the oesophago-gastric junction. The cardiogastric gland adjoins the cardiac area.

The Fundus.—The gastric glands in the fundus are much shorter and more coiled than in other regions. The mucosal thickness here is only about one-third that of the corpus and cardiogastric gland. Surface and neck mucous, parietal, chief, and argentaffin cells are all present, but the chief cells are much more abundant, relative to parietal cell population, than in the corpus and cardiogastric gland. Chief cells almost exclusively occupy the basal half of the individual gastric glands, with only occasional parietal cells or mucous neck cells wedged in among them. The luminal half of the glands is composed of surface mucous cells.

The Corpus.—This region is composed of long, straight, simple gastric glands. They are densely packed, parallel to each other, and perpendicular to the muscularis mucosae. The glands contain all five major cell types, and

and empty into gastric "pits" or foveolae. The distribution of cells and thickness of mucosa closely approximates the situation in the cardiogastric gland, and will be described under that heading. The only difference between this region and the cardiogastric gland is that here the mucosa forms a simple lining sheet without convolutions and impocketings. The corpus mucosa blends gradually into the mucosa of other regions.

The Cardiogastric Gland.—The mucosa of the cardiogastric gland is folded upon itself in an elaborate manner, so that sac-like impocketings result. The nature of these impocketings is best understood by sections through the edge of the cardiogastric gland, where the mucosa is beginning to fold under itself (Pl. III, Fig. 3). The sacs thus formed are simple, branched, tubular, and do not anastomose. Muscularis mucosae closely invests these sacs and their branches. The ostia of the sacs are 1–5 mm. across, and represent the 15–30 crater-like openings seen macroscopically. Because of the large volume occupied by the impocketings relative to the smaller area required for their ostia, the underlying mass of sacs bulges beyond the outer rows of ostia. Thus it is that the peripheral rim of the cardiogastric gland underlies a simple epithelial sheet that does not bear openings (Pl. III, Fig. 3). As Fig. 3 also shows, the submucosa sweeps up among the sacs, bringing in its areolar tissue nerve, lymph and blood supply to the sacs, as well as some smooth muscle slips which are probably derived from the muscularis mucosae and externa. The muscularis and serosal coats simply pass underneath the whole mucosal mass, without investing individual sacs. The collection of longitudinally oriented striated muscle fibres, just deep to the serosa, is easily seen histologically (Pl. III, Fig. 4). For a more detailed description of the muscularis layers the reader is referred to Johnstone's account (1898).

A section cut transversely across the lesser curve (the axis of the gland) may show a bifid ridged median leaf of submucosa, with no underlying sacs, and correspondingly thickened submucosa and muscularis layers (Pl. III, Fig. 4).

The mucosa of the gland is similar to that of the corpus in cytological organisation. While its single-sheet thickness (0·63 mm.) is approximately that of the corpus, the mucosa is piled up so that the mean total thickness of mucosa in a given section is four to ten times that of any other stomach region.

The individual glands are simple, straight and densely packed, opening near the lumen of the stomach into foveolae (Pl. IV). These contain the five principal cell types found in other mammalian stomachs. The chief cells are concentrated in the basal fifth of the glands, with occasional parietal cells wedged among them. A few argentaffin cells are near the base of some of the glands. A large number of parietal cells are present in every gland in its middle three fifths with mucous neck cells or, occasionally, surface mucous cells interspersed. Approximately 500 cells constitute a single gland.

Connective tissue, capillaries, fibroblasts, and a few smooth muscle fibres fill in the spaces between glands. The capillary plexus is particularly rich in the muscularis mucosae and near the gastric foveolae, just beneath the surface mucous cells. Large, thin-walled vessels, packed with blood cells, are often seen between foveolae.

The Pylorus.—The mucosa of the corpus gradually loses its parietal and chief cells as the pyloric opening is approached, although argentaffin cells remain. The epithelium becomes thinner, and foveolae eventually extend halfway to the muscularis mucosae. The glands become entirely composed of mucus-secreting and argentaffin cells. There is an intermediate zone, approximately 2 cm. wide, containing both corpus glands and pyloric glands. This zone begins at the pyloric edge of the cardiogastric gland and continues around towards the greater curve. Near the pylorus the glands become widely spaced, and considerable connective tissue, fibroblasts, and smooth muscle fills the spaces between glands. The glands themselves are coiled and may show simple branching. A suggestion of villi is seen in the few millimetres immediately surrounding the pyloro-duodenal junction, with several gland foveolae opening into each villus.

Cytology of the Gastric Glands.—Except where stated otherwise, these observations apply equally to cardiogastric gland and other regions of the stomach. Five distinct mucosal cell types are evident; surface mucous, neck mucous, parietal, chief and argyrophilic cells.

Surface Mucous Cells.—Surface mucous cells line the foveolae of the gastric glands and bridge the mucosa between the foveolae. They are tall, columnar cells, with a height of 15 to 20 microns and a width of about four microns. The nucleus is spherical and basally or centrally placed. A large nucleolus may be present. These cells are easily identified in PAS sections (Pl. v, Fig. 1) by the heavily striated mucous granules in the cytoplasm. These granules are densely packed and are present in the apical half of the cell. They do not encroach on the nucleus so as to flatten it, and usually a clear zone is seen between nucleus and mucus accumulations.

Neck Mucous Cells.—Neck mucous cells are found among the parietal cells along the elongated neck of the glands. They are low columnar or cuboidal and about 5μ in diameter. The shape of these cells is variable, and the cell may have a broad base and narrow apex, or vice versa. The nucleus is basally located and flattened by an encroachment of mucous granules on its apical side. Sometimes PAS-positive material is found on the basal side of the nucleus.

Chief Cells.—Chief cells are localised in the basal regions of the gastric glands, and stain readily with basic dyes. They are cuboidal or low columnar, and measures $5–10\mu$ in diameter. The nucleus is spherical and centrally located. Accumulations of chromophil substance are present in the basal part of the cell. This substance is distributed in parallel, concentric or radially oriented rows, which give the cytoplasm a clearly striated appearance (Pl. v, Fig. 2). "*Nebenkerns*" (concentric spirallying ergastoplasmic rings) are clearly evident in some cells. The striations often seem centered about or actually attached to the nuclear membrane. The thickness of each striation is 0·2 microns or less. Accurate measurement is not possible because of the limited resolving power of the light microscope.

The apical portion of the cell has a pale, bubbly or frothy appearance suggestive of the zymogen granules characteristic of these cells. The vacuolations are spherical and are about 1μ in diameter. The top of the cell is frequently seen bulging into the lumen of its gland tubule. This distension is evidently caused by local accumulation of the secretory product. In certain regions of the mucosa most chief cells are seen to be almost full of the zymogen granules, with the ergastoplasm confined to the extreme base of the cell. In other mucosal regions the reverse is true, with ergastoplasm occupying the entire cell.

Parietal Cell.—Parietal cells are easily distinguished by their large size (diameter $10–25\mu$), and acdiophilic cytoplasm. They are confined mainly to the middle three fifths of the gastric glands, but occasionally wedge among both surface mucous cells and the basally located chief cells. Parietal cells appear to have a slightly larger mean diameter in the cardiogastric gland than in other stomach regions, although this has not been statistically verified. Parietal cells are spherical or pyramidal in shape. The nucleus is large, centrally located and may contain a nucleolus. The cytoplasm has a definite granular appearance. An intracellular canaliculus is present, staining negatively in haematoxylin-eosin preparations. The canaliculus is sharply outlined occasionally in PAS-treated material, as PAS-positive substances in the canaliculus cause a definite purplish ring (Pl. v, Fig. 3). This canaliculus surrounds the nucleus in a nearly completed "horseshoe", the open ends of which join the lumen of the gastric gland. The canaliculus follows a course midway between nuclear membrane and external limiting membrane.

The Argyrophilic Cell.—Argentaffin and other argyrophilic cells are present throughout the stomach mucosa, though not in great numbers. Both are demonstrated by the Masson stain. When present, they are usually basally located in the gastric glands. Their size is difficult to measure, owing to the great variety of shapes they assume (Pl. v, Fig. 4). Some cells are spherical and are about 10μ in diameter, others are greatly elongated (up to 30μ in length), with a narrow width (5μ) corresponding to the diameter of the nucleus. The nucleus itself is spherical and may be centrally placed in some cells. Not infrequently the nucleus may be centered at one end of the cell, with most of the cytoplasm concentrated around it. In such cases a long tapering extension of cytoplasm extends in one direction away from the nucleus, thus giving the cell a flask or club-shaped appearance. This extension may narrow considerably until it resembles a thread-like neuron process, 0·2μ in diameter. The argyrophilic cell seems to adapt its flexible shape to directly conform with its neighbours, and does not necessarily abut on the lumen of the gland tubules. The cytoplasm contains hundreds of small granules which may be stained by silver impregnation methods. Many of these granules are attached to the nuclear membrane.

Nervous Tissue.—Anterior and posterior vagal trunks are present on the oesophagus in its lower portion. These send branches to the cardiogastric gland as well as to the remainder of the stomach. A definite "spray" of fibres is often seen entering the cardiogastric gland.

Ganglia and nerve fibres were poorly or incompletely demonstrated in haematoxylin-eosin and the various special stains which it was hoped would distinguish them. However, the existence of the myenteric plexus of Auerbach in the muscularis externa was clearly demonstrated, with both nerve fibres and large multipolar neurons present. The submucous plexus of Meissner was not clearly seen.

Discussion

Some earlier observers, as well as the present authors, have seen that the cardiogastric gland of the wombat is not histologically a separate organ from the stomach (Oppel, 1896; Johnstone, 1898). Its mucosa is composed of the same types of secretory cells which are found in other stomach regions and in other mammalian stomachs. The similarities of thickness and cell distribution in corpus mucosa and cardiogastric gland mucosa do not suggest a unique function attributable to the cardiogastric gland.

The parietal cells of the wombat cardiogastric gland appear larger than those elsewhere in the stomach, an observation which had been previously made in beaver stomach (Nasset, 1953). However, we cannot verify in the wombat any increased height of mucosa or density of parietal cells in the cardiogastric mucosal lining as compared to neighbouring mucosa in the stomach body; such an increase has been reported in the beaver (Nasset, 1953). No comparisons based on those parameters have been reported for the koala stomach. It must be recognised that the total amount of enzyme and acid-secreting mucosa in the gland represents a very considerable fraction of the total stomach mucosa. While the localisation of this mucosa has been carried to an extreme in the wombat, koala and beaver, regional concentrations of parietal cells have been observed in other mammals. In man, dog, cat and rat the greatest density of parietal cell population exists in the stomach body on the greater curve (Oi *et al.*, 1958). The rabbit has the greatest concentration in the fundus, while the guinea pig has its greatest parietal cell concentration in the same site on the lesser curve as does the wombat (Oi *et al.*, 1958). The guinea pig does not possess a cardiogastric gland. Of interest is the fact that in human embryos gastric pits and differentiating gastric epithelial cells are first seen along the lesser curve, particularly in the oral part (Salenius, 1962).

The subcellular features of the mucosal cells in the cardiogastric glands or in other regions of the stomach were not unusual. However, the clarity and organised patterns of the ergastoplasmic striations in the chief cells were of some interest and are believed to be a well-defined rough endoplasmic reticulum. These striations have been described in the chief cells and pancreatic acinar cells of other mammals (Hagenau, 1958; Dalton, 1951).

Johnstone (1898) and Oppel (1896) have speculated about the significance of the cardiogastric gland in mammalian evolution and the genetic differences which may underlie its development. Oppel believed that the glandular apparatus of the wombat and koala blossomed into the cardiogastric gland in order to facilitate assimilation of large amounts of food. Thus the gland is viewed as an accessory structure which in practice augments the limited circumference of the stomach and represents a beneficial evolutionary development. It is not certain, however, in what way the digestion of large amounts of food is particularly enhanced by this gland. We have obtained considerable quantitative data on the acid, enzyme, and electrolyte composition of sections from the cardiogastric gland. Further discussion of the physiologic role of this anatomical specialisation is put forward by Milton, Hingson and George (1967).

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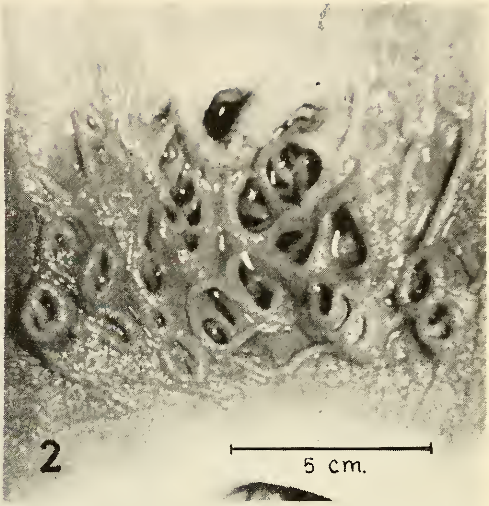

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The mucosa of the stomach of the wombat.